The role of insulin receptor substrate (IRS) proteins in oncogenic transformation

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Abstract: Insulin Receptor Substrate (IRS) proteins are the main cytoplasmic adaptor molecules involved in transducing extracellular signals from receptors to downstream proteins. This protein family have pivotal roles on maintenance, distribution and regulation of signaling networks. Since IRS1/2 interact with and transmit signals from the receptors of insulin, Insulin Like Growth Factor 1 (IGF1), prolactin, growth hormone (GH), leptin, Vascular Endothelial Growth Factor (VEGF), TrkB, ALK and integrins this promoted scientist to think that IRS1 may have functions in cell proliferation, tumorigenesis and metastasis. Therefore, over the past decade, studies on IRS proteins and their functions in cancer has been increased and these studies provided valuable results claiming the involvement of IRS1/2 in cancer development. In this review, we discuss the function and contributions of IRS1 and IRS2 in development of breast cancer.

Key words: Breast Cancer; Insulin Receptor Substrate Protein; Insulin Signaling; Insulin Receptor Substrate 1; Insulin Receptor Substrate 2.

Introduction

Insulin is a primary anabolic hormone responsible for maintenance of metabolic homeostasis (1,2). Insulin and insulin-like growth factors (IGF1 and IGF2) regulate anabolic and catabolic reactions through highly complex signaling networks (3). During insulin signaling, ligand-bound insulin receptor (IR) or insulin like growth factor receptor (IGF-1R) are phosphorylated at Tyrosine residues via auto-phosphorylation, then, IRS proteins bind to these phosphotyrosines through their SH2 domains. IR-bound IRS is phosphorylated by IR at YXXM motifs, four of which were localized at the C-terminal. These phosphorylated motifs create docking sites for PI3Kp85α, and YXXM-bound PI3Kp85α binds to PI3Kp110α and activates it. Activated PI3Kp110α generates PIP3 from PIP2, and therefore, activates PDK1. Activated PDK1 activates AKT by phosphorylating it at Thr308. Activated AKT regulates glucose-uptake by phosphorylating glucose transporters GLUT1 and GLUT4 (4,5). Therefore, activation of AKT is primarily responsible for the metabolic functions of insulin. IRS1/2 also activate ERK pathway which regulates the expression of genes whose products control cell proliferation and differentiation (6). (Figure 1)

In addition to being essential for glucose homeostasis, long term activations and overexpressions of IR/IGFR are strongly correlated with hiperplasia, tumor growth and poor prognosis (7-9).

In this article, we will review the recent literature, discuss the results of human and animal model studies, and explain the molecular mechanism and function of IRS proteins in development of breast cancer.

IRS proteins structure

IRS proteins are adaptor proteins and play central role in transmission of signals from multiple receptors and they elicit this through multiple phosphorylation motifs (10).

IRS family consists of four proteins (IRS1-IRS4) with two evolutionary well conserved interaction domains (2,10) (Figure 2). The first one is pleckstrin homology (PH) domain located at N-terminal of the protein and consists of positively charged aminoacids that mediates the interaction of IRS proteins with cell. This domain also leads to diversity of signaling by interac-
tions (9,20). Studies focus on IRS1 and IRS2 proteins and their functions (18,19). Owing to their expression profiles, most of the IRS1 is expressed only in rat not in human. Unlike IRS1 and IRS2 proteins, IRS4 has been ubiquitously expressed in human tissues. IRS3 expression is restricted expression pattern and is found primarily in liver, kidney, thymus and brain (18,19). Owing to their expression profiles, most of the studies focus on IRS1 and IRS2 proteins and their functions (9,20).

Studies on IRS knock-out animal models provided strong evidences on biological roles of IRS proteins. IRS1-/- mice are born 30% smaller than wild type (WT) mice and growth is reduced throughout their lives. These models also have insulin resistance and hyperinsulinemia but they do not develop diabetes and maintain normal pancreatic beta cell numbers (21,22). In contrast to IRS1 -/- model, IRS2 -/- mice have normal size and develop early onset diabetes with decreased beta cell mass. Neuronal proliferation is also decreased in these models and females are infertile (23-25). These phenotypes showed that IRS1 and IRS2 have non-redundant roles even they share significant homology. IRS4 -/- mice are phenotypically normal but have moderate growth retardation, insulin sensitivity defects and reproductive problems (26).

These differences in IRS -/- models especially in somatic growth are also evident in cancer. In addition to these physiological functions of IRS proteins, their interactions with growth factor/hormone receptors such as IGFR, leptin, vascular endothelial growth factors (VEGF), growth hormone, prolactin, integrin, cytokine and interferon receptors in cellular level shows the pivotal roles of IRS proteins in cell proliferation, tumorigenesis and metastasis (16,27-32)(Figure 3).

The roles of IRS1 and IRS2 proteins in developing breast cancer

**IRPs expressions in breast cancer cell lines**

IGF system has an important role in mammary gland development, and several studies have shown the critical roles of IRS1 and IRS2 in normal mammary gland development (33-35). In addition to this, several studies have shown critical roles of IRS1 and IRS2 in development of breast cancer. Transformation capacity of IRS1 protein was first shown Surmacz’ group in mouse embryo fibroblasts (MEFs) (36). Dearth et al. demonstrated that IRS-mediated transformation of mammary epithelial cells in nontransformed human mammary epithelial cells (MCF10A). In this study, they overexpressed IRS1 and IRS2 proteins in MCF10A cells these over-expressions induced proliferations and disrupted the polarity of cells grown in 3D culture (37).

In MCF-7 cell lines, suppression of IRS-1 by siRNA strongly inhibited anchorage-dependent and -independent cell growth and induce apoptotic cell death.
under growth factor- and estrogen reduced conditions (38). On the other hand, MCF7 cells overexpressing IRS1 do not need estrogen for growth and their estrogen requirements were decreased gradually with the level of IRS1 overexpression. Recently a study showed that, knockdown of IRS1 expression by siRNA enhanced tamoxifen induced cell death in MCF7 (39). IRS1 overexpression also promotes growth and proliferation of BT 20 cells, and induced formation of larger tumors in \textit{in vivo} (40). IRS proteins may also be used as prognostic factor in breast cancer. Becker et al. ectopically expressed IRS1 or IRS2 in IRS-null T47D-YA cells and determined their proliferation, motility and global gene expression profile. They also found that IRS1/2 play significant role in induction of TGFβ2, which was claimed to be responsible for IGF1-mediated cell migration. In addition, they also determined level of IRS1 expression in luminal B breast cancer type and found that high expression of IRS1 was correlated with poor relapse-free and overall survival, therefore, they suggested that IRS1 expression could be used as marker to predict response to anti IGF-therapy in breast cancer (41).

\textbf{IRS expressions in \textit{in vivo} studies}

To determine the roles of IRS1 and IRS2 proteins in tumor initiation and progression \textit{in vivo}, researchers generated knock out and transgenic mice with mammary specific overexpression MMTV-HA-IRS1 and MMTV-HA-IRS2 (37). In mice overexpressing IRS1 and IRS2 preneoplastic mammary hyperplasia developed at 24 weeks of age. Multiple mammary gland lesions appeared at 92 weeks in HA-IRS1 overexpressed mice while overexpression of IRS2 caused more rapid tumor formation with a mean time of 68 weeks, these results suggested that multiple and different signaling pathways and oncogenes could be activated by IRS1 and IRS2. In addition to their transformation capacity, overexpressions of IRS1 and IRS2 were detected in 33% and 40% of metastatic lung tumors, respectively (37).

To clarify the molecular mechanisms used by IRS proteins in mammary tumorigenesis, Dr. Dearth et al examined tyrosine phosphorylation of IRS1/2 levels and the activation of downstream elements and found that IRS1/2 were constituatively phosphorylated in mice overexpressing both IRS proteins. Moreover, while ERK1/2 phosphorylation was increased in all tumor cells, phosphorylation AKT was observed in tumors only 10% of animals after infusion of IGF1. These results clearly indicate that activation of ERK and AKT by IRS1/2 is not mediated by a common pathway. This study also showed that in both transgenic mice IRS1/2 interact with and activate β-catenin resulting in induction of expression β-catenin target such as c-myc and cyclin D1 (37) (Figure 3).

In another group of studies Shaw et al. generated polyoma virus middle T:IRS1 null and IRS2 null transgenic mice to identify the specific roles of IRS1/2 in tumor progression and metastases (42-44). Both group of animals developed tumors with the same onset and growth rate, however, unlike the redundant roles of IRS1/2 in tumor growth, they have distinct effects on tumor progression. IRS1 -/- mice showed increase in lung metastases while lung metastases decreased in IRS2 -/- mice. They also showed that ectopic expression of IRS2 compensated the loss of IRS1 in tumor metastases by increasing phosphorylations and activations of AKT/mTOR, but they did not observe IRS1 compensation in IRS2 -/- mice (43). These results suggested that IRS2 is primarily responsible for cell motility and metastases while IRS1 is mainly important for cell proliferation (42-44).

\textbf{IRS expressions in human breast tumors}

After establishment of convincing publications about involvement of IRS1/2 in breast cancer development and metastasis several groups determined expression levels of IRS1 and IRS2 in human breast cancer samples by immunohistochemistry, however, results of these studies produced controversial results. For example, Schnarr et. Al. found that both IGFR and IRS1 expressions were down-regulated in advanced human breast cancer cells whereas Koda et al showed that IRS1 expression was elevated in human breast cancer cells and its lymph node metastases (45,46). Porter et al. also analyzed IRS1 and IRS2 expression in human breast tumor samples and found that IRS1 expression was elevated in localized breast tumors while IRS2 expression was associated with an invasive breast tumors (47). These results are in good agreement with previous publications (42-44) which claimed induction and suppression of breast cancer cells by IRS2 and IRS1, respectively.

\textbf{Conclusion}

IRSs are critical elements and multitask proteins that interacts with a multitude of proteins in tumorigenesis. Although several publications determined expression levels of IRS1 and IRS2 in different type and models of breast cancer samples, we don’t know much about phosphorylation status of IRS1 and IRS2. Also, we don’t know how IRS proteins are localized in different type of breast cancer cells, and whether they use different partners to elicit their functions. In addition to these, it is important to determine the levels of phosphatases which dephosphorylate IRS proteins. Therefore, in addition to expression analysis component of signaling molecules which directly affect modifications and functions of IRS1 and IRS2 must be determined in tumor samples simultaneously.

\textbf{References}


